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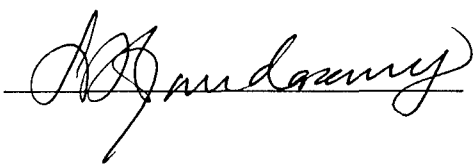

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This report describes preliminary results from our studies at the use of breast cancer DNA Synthesome as an *In Vitro* Model system to evaluate the mechanism of action of anticancer drugs. In this work the pharmacological profile of new eight hydrazone derivatives structurally designed as ribonucleotide reductase inhibitors are discussed. Also a series of new seven 1,4-naphtoquinones derivatives structurally related to the natural product lapachol and designed to act as topoisomerase inhibitors were investigated as well. The antiproliferative activity and the ability to inhibit DNA synthesis through preventing ^{3}H-thymidine incorporation were determined using MCF-7 breast cancer cell lines and some derivatives showed significant results. Our study showed also that the hydrazone new drugs were unable to inhibit the cellular DNA synthetic apparatus in the *in vitro* DNA replication assay using the human breast cancer DNA synthesome multi-protein complex what is consistent with their being RR inhibitors since the deoxyribonucleotide biosynthesis enzymes are not a part of the DNA synthesome. At least four compounds of the 1,4-naphtoquinone series showed remarkable results and seem to be promising drugs for the study of the appliance of the breast cancer DNA synthesome as a model system to evaluate anticancer drugs.

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ABSTRACT

This report describes preliminary results from our studies at the use of breast cancer DNA Synthesome as an *In Vitro* Model system to evaluate the mechanism of action of anticancer drugs. In this work the pharmacological profile of new eight hydrazone derivatives structurally designed as ribonucleotide reductase inhibitors are discussed. Also a series of new seven 1,4-naphtoquinones derivatives structurally related to the natural product lapachol and designed to act as topoisomerase inhibitors were investigated as well. The antiproliferative activity and the ability to inhibit DNA synthesis through preventing ^{3}H-thymidine incorporation were determined using MCF-7 breast cancer cell lines and some derivatives showed significant results. Our study showed also that the hydrazone new drugs were unable to inhibit the cellular DNA synthetic apparatus in the *in vitro* DNA replication assay using the human breast cancer DNA synthesome multi-protein complex what is consistent with their being RR inhibitors since the deoxyribonucleotide biosynthesis enzymes are not a part of the DNA synthesome. At least four compounds of the 1,4-naphtoquinone series showed remarkable results and seem to be promising drugs for the study of the appliance of the breast cancer DNA synthesome as a model system to evaluate anticancer drugs.

INTRODUCTION

Breast cancer is one of the commonly diagnosed female cancers and the second leading cause of cell death among women (Weinberg, R. A., 1996). Besides that, the treatments traditionally used in the cancer treatment obtained positive results only in a small percentage of solid tumors and resulted in undesired side effects (Alessandro, R. *et al.*, 1996; Hellman, S., Vokes, E. E., 1996). Clarification of the mechanism of action of chemotherapeutic agents at the cellular and molecular levels is crucial to the development of more effective and rational breast cancer treatment and more specific drugs with less toxicity (Weinberg, R. A., 1996; Alessandro, R. *et al.*, 1996; Barinaga, M., 1997; Fearon, E. R., 1997). Our research group was the first to successfully isolate and purify a functional multiprotein DNA replication complex from human breast cancer cells, which was designated the DNA synthesome (Malkas, L. H. *et al.*, 1990; Wu, Y. *et al.*, 1994; Applegren, N. *et al.*, 1995; Simbulan-Rosenthal, C. M. *et al.*, 1996; Coll, J. M. *et al.*, 1996; Lin, S. *et al.*, 1997; Coll, J. M. *et al.*, 1997; Sekowski, J. W. *et al.*, 1998; Sekowski, J. W. *et al.*, 1998; Bechtel, P. E. *et al.*, 1998; Simbulan-Rosenthal, C. M., 1998). The discover and characterization of the DNA synthesome lead us to the development of a new and powerful *in vitro* replication system DNA synthesome-driven. This new model can be used to study the mechanism of action of anticancer drugs which directly affect the DNA synthesis (Wills, P. *et al.*, 1996; Coll, J. M. *et al.*, 1996) and will greatly facilitate the development of improved anti-breast cancer drugs.

As part of a research program aiming at the appliance of the breast cancer DNA synthesome as model system for studying the mechanism of action of anticancer drugs and while searching for new compounds presenting an antiproliferative activity on breast cancer cells we report the preliminary pharmacological evaluation of two new series of antiproliferative compounds.

One of this new series investigated are hydrazones derivatives (Figures 1) compounds LASSBio 466,467, 431,276, 273 and 578 (Lima, L. M. *et al.*, 1999; Lima, P. C. *et al.*, 1999; de Souza, A. F. *et al.*, 1998; de Souza, A. F. *et al.*, 1999) (Figure 1), structurally designed to act as ribonucleotide reductase (RR) inhibitors. The sulfonylurea derivatives LASSBio 378 and LASSBio215 (Figure 1) were also evaluated (Figures 2 and 3 and Table 1) in order to investigate the contribution of the sulfonylthiourea and sulfonamide urea units to the antiproliferative activity. The therapeutic benefits of inhibitors of the enzyme ribonucleotide reductase (RR) (Cory, J. G., *et al.*, 1983; Smith, E. L. *et al.*, 1985) have been well established in many neoplastic diseases. A classic example is the antitumor drug hydroxyurea which blocks the DNA replication via the inhibition of the RR activity (Navarra, P., Preziosi, P., 1999). These therapeutic effects are based on the important role played by the RR which catalyzes the conversion of the ribonucleotides into deoxyribonucleotides in the cytoplasm (Engstrom, Y. *et al.*, 1988) and it is considered the rate-limiting step of DNA synthesis and hence cell proliferation (Elford, H. L. *et al.*, 1970; Weber, G., 1977; Cory, J. G., Sato, A., 1983).

We decided also to evaluate a new series of 1,4-naphtoquinones derivatives structurally related to the natural product lapachol. 1,4-naphtoquinones are widespread in nature and have been isolated from plants and exhibit interesting biological activities (Thomson, R. H., 1971; Morton, R. A., 1965). Lapachol (2-hydroxy-3-prenyl-1,4-naphtoquinine), found in species of *Tabebuia*, was shown to be active against Walker-256 carcinoma and Yoshida sarcoma (Driscoll, J. S., *et al.*, 1974; Rao, K. V., *et al.*, 1968). Several synthetic derivatives of lapachol have been prepared and showed to be active in leukemia (da Consolacao, M., *et al.*, 1975), and in a panel of solid tumors *in vitro* (Ueda, S., *et al.*, 1994; Saizarbiotoria, T. C., *et al.*, 1992; Kingston, D. G. *et al.*, 1980).

BODY

Investigation on the activity of the hydrazone series in breast cancer cells

Materials

The drugs tested LASSBio 466, 467, 276, 273, 378, 431, 578 and 215 were supplied by Dr Eliezer J. Barreiro, Laboratorio de Avaliacao e Sintese de Substancias Bioativas (LASSBio), University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil. These derivatives were synthesized in this laboratory as described previously (Lima, L. M. *et al.*, 1999; Lima, P. C. *et al.*, 1999; Souza, A. F. *et al.*,

1998; Souza, A. F. *et al.*, 1999). The drugs were dissolved in dimethyl sulfoxide (DMSO) and prepared as 1M, 100 mM and 1mM stock solutions and stored at -5°C.

The hydroxyurea and araCTP (Sigma) were dissolved in deionyzed water and prepared as 1M and 100mM stock solutions and stored at -5°C.

In the assays using MCF-7 cells we used MEM medium (GibcoBRL), 1mM sodium pyruvate (90 %) (GibcoBRL) supplemented with 10% fetal bovine serum, 1 % penicillin/streptomicin (will be referred to as complete medium). The drug solutions diluted with the appropriate medium were sterilized by passing through syringe filter (0.2 μm) (Nalgene). The concentration of DMSO used in the final solutions had no effect on cellular proliferation, [^{3}H]thymidine incorporation in the DNA or *in vitro* SV40 DNA replication observed in the respective assays.

Cell Proliferation Assay

The effect of the test-compounds on MCF-7 cellular proliferation was examined using the MTT colorimmetric assay (Mossmann, T., 1983; Denizot, F. & Lang, R., 1986; Barile, F. A., 1994).

The MCF-7 cells were seeded in 96-well flat bottomed dishes (Falcon, Becton Dickinson Lab-ware) at 1.2-3.2 x 10^4 cells/mL (100 μL). Twenty four hours later, when the cells were in exponential growth, the test-compounds were added at different concentrations. Each drug concentration was tested in four separate wells and at least five different concentrations of each drug were used to determine the IC_{50} as a mean of at least two separate experiments.

The cells were incubated for 24, 48, 72 and 96 hours in the absence and presence of the test-compounds. After the incubation period, the medium was removed and replaced with a 1mg/mL (1mL) MTT solution in MEM without bovine fetal serum or antibiotic. The plates were incubated at 37°C for 3 hours.

At the end of the incubation period the untransformed MTT was removed and DMSO was added to each well. The plates were then vigorously shaken for 5 minutes and the absorbance of each well was measured at 560 nm a 96-well plate reader (Victor 1420 Multilabel Counter - Wallac). The absorbance values were corrected for by preparation of blank wells containing all additions apart from the cells. The results were expressed as the means of 4 replicates. The inhibitory concentration value (IC_{50}) was defined as the concentration of drug that reduced absorbance to 50 % of that found for the untreated control 70 hours after adding the compounds. The IC_{50} was determined mathematically based on the Hill coefficient (Figure1, table 1).

The cytotoxic effect of DMSO was obtained previously at the same assay conditions.

Some structure-activity analysis could be concluded from this experiment. Table 1 and Figure 1 demonstrates that all of the hydrazone analogues inhibited the cell proliferation in some extent. The acylhydrazone derivatives LASSBio 466 and 467 inhibited the MCF-7 cell proliferation (IC_{50} = 3.5 and 8.9 μM) to a greater

extent than the sulfonyl hydrazone compounds (LASSBio 273, 276 and 578) in the same experiments (IC_{50} = 121.4, 145.4 and ≥ 300 μM). The acylhydrazone compound LASSBio 431 (IC_{50}= 283.9 μM) which doesn't have the OH aromatic substituint, as expected, showed less activity than the analogues 466 and 467. Curiously, LASSBio273, which can coordinate with intracellular iron only in a bidentate manner, showed a compared activity with LASSBio276 in this assay. On the other hand, the results obtained with the compound LASSBio578 showed that the introduction of a hydroxylic substituint in the 2 position of the aromatic ring leading to the formation of a OH*-N*-O* tridentate ligand system resulted in a loss of activity when compared with the analogs LASSBio 273 and 276.

These results confirm that similar to the compounds pyridoxal isonicotinoyl hydrazones (Richardson, D. R., Milnes, K., 1997) and thiosemicarbazones (Antonini, I. *et al.*,1981) the presence of a tridentate ligand ssystem X*-N*-Y* is important for the antiproliferative activity as also for the RR inhibition at least in the acylhydrazone series. The small difference between the IC_{50} data obtained for LASSBio466 and LASSBio467 for the cell lines tested indicates that probably there is no conformational effects interfering in the coordination geometry of this series of compounds.

In order to investigate the possible pharmacophoric contribution of the acylhydrazone moiety of LASSBio 466 and 467 and the structural related sulfonylhydrazone bioisoster moiety of LASSBio 276, 273 and 578, the sulfonamide urea compound LASSBio 378 was also screened against human breast cancer MCF-7 cells in culture as shown in Table 1 and Figure 1. The compound LASSBio 378 showed a reduced activity (IC_{50} > 300 μM) indicating that the replacement of the sulfonylhydrazone subunit of LASSBio 273 and 276 by the sulfonylthiourea moiety lead to a loss of activity.

Table 1: Inhibition of cell growth and ^{3}H-thymidine incorporation into DNA by several hydrazone analogues.

Compounds	IC50 (μM) MCF-7 cell proliferation[a]	[^{3}H]thymidine incorporation MCF-7[a]
Hydroxyurea	nd[b]	319.3 ± 39.35
LASSBio466	3.52 ± 0.23	3.55 ± 0.92
LASSBio467	8.96 ± 0.63	8.14 ± 1.81
LASSBio431	283.9 ± 16.83	130.6 ± 9.12
LASSBio 276	145.4 ± 36.87	68.75 ± 2.74
LASSBio273	121.4 ± 13.42	58.86 ± 5.17
LASSBio578	≥ 300	126.5 ± 3.17
LASSBio378	> 300	147.9 ± 19.28
LASSBio215	> 300	251.1 ± 32.64

[a] Mean of at least two independent experiments which four replicates determinations were taken within each experiment ± standard error. [b] nd= not determined.

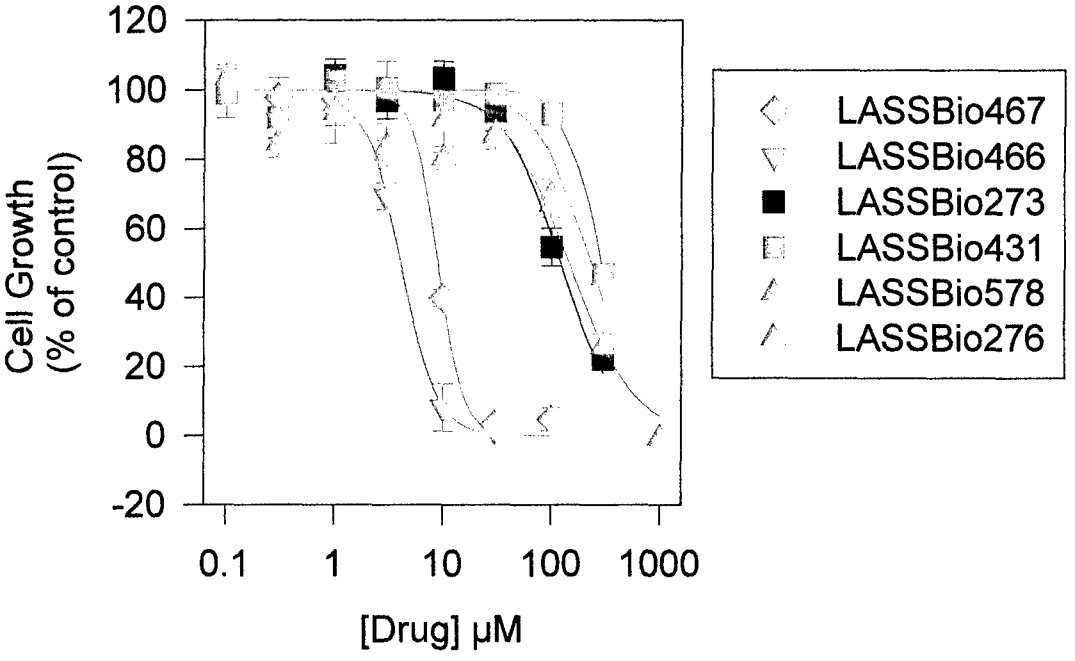

Figure 1: Effect of the hydrazone derivatives on intact MCF-7 cells proliferation after 70 hours of treatment with the drugs. Each point represents the average of at least two independent experiments in which four replicates determinations were taken; error bars represent the standard errors of the means. Cells grown in the absence of drug served as the controls (100 %) to which the drug-treated cells were compared. The IC_{50} curves were determined mathematically based on the Hill coefficient.

[methyl-^{3}H]thymidine incorporation assay

The synthesis of DNA was measured indirectly by measuring [methyl-^{3}H]thymidine incorporation. The effects of the compounds were examined by seeding MCF-7 cells in 24-well plates (Falcon, Becton Dickinson Lab-ware) at 1.0 x 104 cells/well in 1 mL of MEM complete medium. The cells were incubated at 37 °C, 5% CO2 and 95 % air for around 24 hours and then 1 mL of complete medium containing the test-compounds at a range of concentrations were added. Each concentration of drug was tested in four separate wells, in at least five concentrations of each drug and in two separated experiments. The inhibitory concentration value (IC_{50}) was defined as the concentration of drug that reduced

tritium incorporation to 50 % of that found for the untreated control 70 hours after adding the compounds. The IC_{50} value was determined mathematically based on the Hill coefficient and is showed in Figure 2 and Table 1.

The cells were incubated for 24, 48, 72 hours after the addition of the drugs. At the end of the period of incubation the cells were labeled with [methyl-^{3}H]-thymidine (1mCi/mL) (New England Nuclear, Boston, MA) at 1 μCi/well for 1 hour at 37 °C. Then the cells were assayed for incorporation of tritium into trichloroacetic acid preciptable material deposited onto GF/C glass fiber filters (25 mm) (Whatman) held in vacuum manifold. The filters were washed with 10 % trichloroacetic acid and 95% ethanol and placed into scintillation vials. Three mL of scintillation fluid Bio-Safe NA (Research Products International Corp., Illinois) was added and the radioactivity per vial was counted on a scintillation counter (Liquid Scintillation Analyzer, Tri-Carb, Packard).

The profile of inhibition obtained in the antiproliferative assay was confirmed in the radioactive assay where the acylhydrazone derivatives LASSBio 466 and 467 were the more potent compounds (IC_{50} = 3.5 and 8.1 μM) (Table 1, Figure 2) followed by the sulfonylhydrazone derivatives LASSBio 276 and 273 (IC_{50}= 68.75 and 58.86 μM). The hydrazone compounds showed greater activity when compared to the RR inhibitor hydroxyurea (IC_{50} > 300 μM). The compounds, which had the hydrazone subunit, replaced by a sulfonylthiourea moiety (LASSBio378) or a sulfonamide urea moiety (LASSBio215) showed a loss of activity as showed in Table 1 and Figure 2.

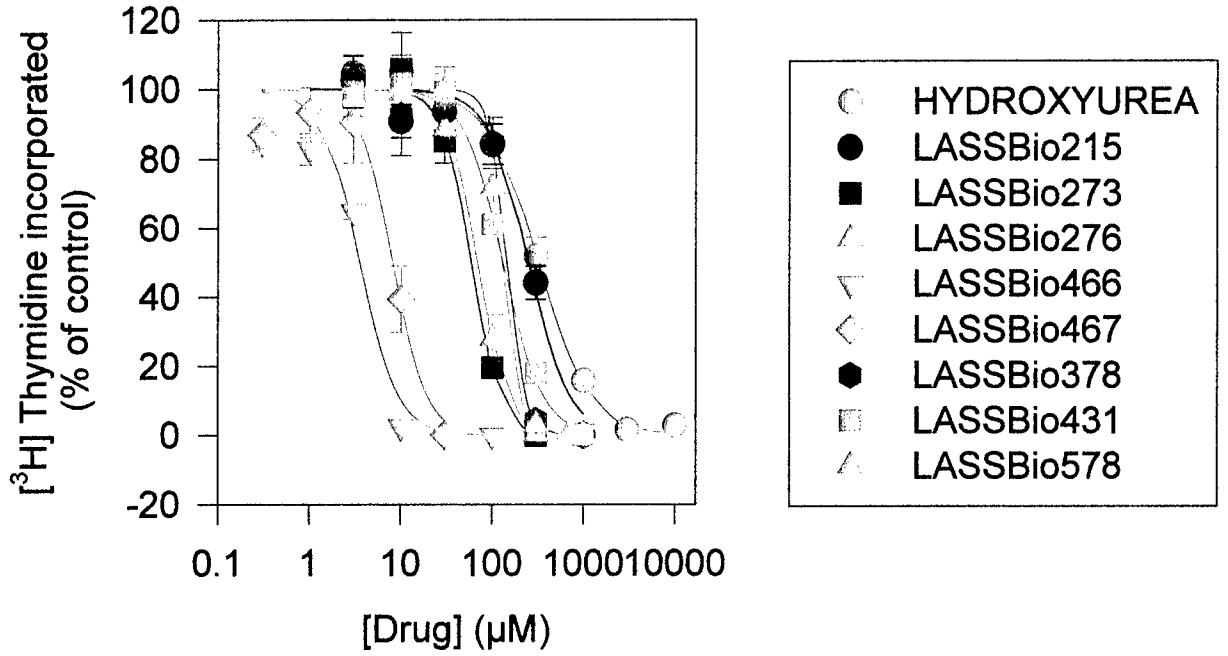

Figure 2: Effect of the hydrazones analogues and hydroxyurea on intact MCF-7 cell DNA synthesis after 70 hours of treatment with the drugs. Each point represents the average of at least two independent experiments in which four replicates determinations were taken; error bars represent the standard errors of the means. Cells grown and labeled in the absence of drug served as the controls (100 %) to which the drug-treated cells were compared. The IC_{50} curves were determined mathematically based on the Hill coefficient.

Isolation of the MDA-MB486 DNA synthesome

MDA-MB486 (7.4 g) cells were homogenized, and the breast cell DNA synthesome was purified according to our previously published procedure (Coll, J. M. *et al.*, 1996). Briefly, the respective cell pellet was resuspended in one volume of buffer (200mM sucrose, 50 mM HEPES (pH 7.5), 5 mM KCl, 5 mM MgCl, 2 mM DTT, 0.1 mM PMSF) and homogenized using a Dounce homogenizer. The homogenate was then fractionated into a nuclear pellet and a cytosolic extract. The nuclei was extracted with a high salt buffer (2M KCl), while the cytosolic fraction was used to prepare a postmicrosomal supernatant (S-3). The nuclear extract and the postmicrosomal supernatant were combined and

adjusted to 2 M KCl and 5 % polyethylene glycol. The mixture was rocked for 1 h at 4 °C, then centrifuged at 5,000 rpm for 15 min (4 °C). The resulting supernatant was then dialyzed against a buffer containing 0.25 M sucrose. The dialyzed fraction was clarified by centrifugation at 15,000 rpm for 15 min, and the supernatant solution was layered onto a 4 mL 2 M sucrose cushion containing the dialyzes buffer. After centrifugation at 40,000 rpm overnight (4 °C) the supernatant S-4 and sucrose interface P-4 fractions were collected and dialyzed against Kenny's buffer (50 mM Hepes, pH7.5, 5 m m KCl, 10 % glycerol) containing 1 mM DTT. The fractions were then immediately tested for *in vitro* SV40 DNA replication activities.

In vitro SV40 replication assay

The assay was performed essentially as described previously (Malkas, H. *et al.*, 1990) in the absence and presence of different concentrations of drug. Assay reaction mixtures (12.5 μL) contained 30 mM HEPES (pH 7.5); 7.5mM $MgCl_2$; 0.5 mM DTT, purified SV40 large T-antigen, 24.8 ng plasmid $pSVO^+$ containing an insert of SV40 replication-origin DNA sequences; 100 μM each dTTP, dATP, dGTP; 200 μM each rCTP, rGTP, UTP; 4mM ATP; 25 μM [α-^{32}P]dCTP; 40 mM creatine phosphate; 1 μg creatine kinase. Each reaction was incubated for 4 h at 37 °C. The replication assay reaction products were processed using DE81 (Whatman, Maidstone, UK) filter binding to quantitate the amount of radiolabel incorporated into the replication products. The results are showed in Figure 3.

Our results showed that most of these new drugs were unable to inhibit the cellular DNA synthetic apparatus at concentrations up to 300 μM as shown in Figure 3. This is consistent with their being RR inhibitors since the deoxyribonucleotide biosynthesis enzymes are not a part of the DNA synthesome.

In order to investigate the mechanism of action of this new drugs we had begun to develop RR purification and assay to test this compounds.

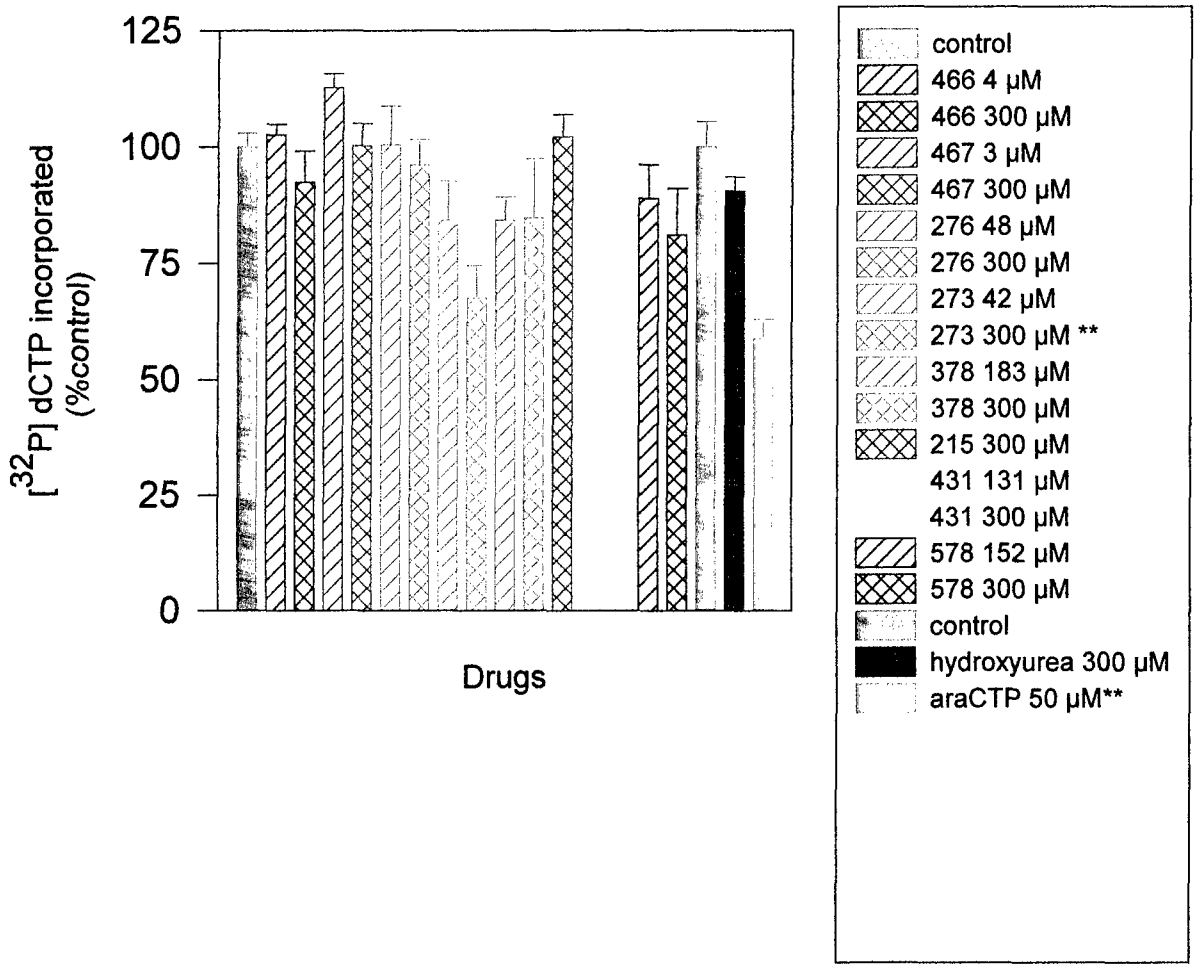

Figure 3: Effect of the hydrazone derivatives, hydroxyurea and araCTP on DNA synthesome-mediated *in vitro* SV40 DNA replication. The assays were performed as described above. Each point represents at least two independent experiments in which four replicates determinations were taken; error bars represent the standard errors of the means. Control reactions were performed in the absence of drug (100 %).

** P< 0.001 compared to appropriate control (ANOVA followed by Dunnett test).

Investigation on the activity of the 1,4-napthtoquinone series in breast cancer cells

The drugs tested PCALC8, 12, 13, 17, 22, 23 and 32 were supplied by Dr Paulo R. R. Costa, Laboratorio de Quimica Bioorganica (LBQ), NPPN, University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil. These derivatives were synthesized in this laboratory. The drugs were dissolved in dimethyl sulfoxide (DMSO) and prepared as 1M, 100 mM and 1mM stock solutions and stored at -5°C.

Cell Proliferation Assay

The effect of the test-compounds on MCF-7 cell proliferation was examined using the MTT colorimetric assay (Mossmann, T., 1983; Denizot, F. & Lang, R., 1986; Barile, F. A., 1994).

The MCF-7 cells were seeded in 96-well flat bottom dishes (Falcon, Becton Dickinson Lab-ware) at 1.4 x 10^5 cells/mL (100 µL) on day zero. Twenty four hours later, when the cells were in exponential growth, several concentrations of the test-compounds were added.

The cells were incubated for 24 and 48 hours in the absence and presence of the test-compounds and submitted to the same procedures described above. The inhibitory concentration value (IC_{50}) was defined as the concentration of drug that reduced absorbance to 50 % of that found for the untreated control 24 hours after adding the compounds. Each drug concentration was tested in four separate wells and at least five different concentrations of each drug were used to determine the IC_{50}. The IC_{50} values were determined for each drug as a mean of at least three separate experiments and mathematically based on the Hill coefficient (Figure 4, Table 2).

The results indicated that the compounds PCACLC23 and PCALC12 have comparable and significant activity and are the most potent antiproliferative inhibitors in this serie (Table 2, Figure 4). About 5.34 µM of PCACLC23 and 7.77 µM of PCALC12 were required to reduce MCF-7 cell proliferation to 50 % of the control activity measured in the absence of drug in only 24 hours of treatment of the cells. The MCF-7 cells wre also inihbited by compounds PCALC22 and PCALC8 but they were less effective at inhibiting cell proliferation than compounds PCACLC23 and PCALC12. The IC_{50} values for PCALC22 and PCALC8 in this assay were 27.7 and 29.04 µM, respectively. The other compounds studied in these serie were also able to inhibit cell growth in a concentration-dependent manner but much less efficiently. Unfortunatly, their effect in higher concentrations could not be studied due to their poor solubility in DMSO and aqueous solutions.

Table 2: Inhibition of cell growth and ^{3}H-thymidine incorporation into DNA by several 1,4-naphtoquinones compounds.

Compounds PCALC	IC_{50} (μM) MCF-7 cell proliferation[a]	[^{3}H]thymidine incorporation MCF-7[b]
22	27.7 ± 3.44	3.25 ± 1.28
13	> 100	nd[c]
17	> 100	nd
8	29.04 ± 1.23	5.23 ± 0.95
23	5.34 ± 0.71	12.86 ± 2.47
12	7.77 ± 0.64	3.89 ± 0.73
32	115.3 ± 20.54	nd

[a] Mean of at least three independent experiments which four replicates determinations were taken within each experiment ± standard error. [b] Mean of at least one independent experiments which four replicates determinations were taken within each experiment ± standard error. [c] nd= not determined.

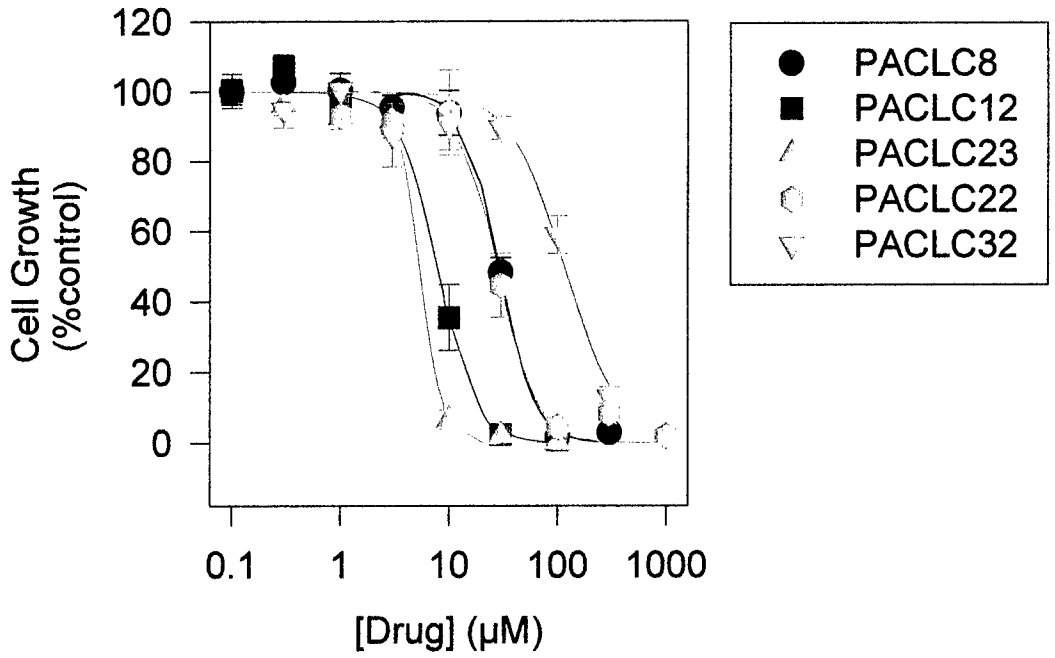

Figure 4: Effect of the 1,4-naphtoquinones derivatives on intact MCF-7 cells proliferation after 24 hours of treatment with the drugs. Each point represents the average of at least three independent experiments in which four replicates determinations were taken; error bars represent the standard errors of the means. Cells grown in the absence of drug served as the controls (100 %) to which the drug-treated cells were compared. The IC_{50} curves were determined mathematically based on the Hill coefficient.

[methyl-^{3}H]thymidine incorporation assay

The synthesis of DNA was measured as described above. The cells were incubated for 24 and 48 hours after the addition of the drugs. Each concentration of drug was tested in four separate wells, in five concentrations of each drug and in at least one experiment. The inhibitory concentration value (IC_{50}) was defined as the concentration of drug that reduced tritium incorporation to 50 % of that

found for the untreated control 24 hours after adding the compounds. The IC_{50} value was determined mathematically based on the Hill coefficient and is showed in Figure 5 and Table 2.

The effect of the 1,4-naphtoquinones derivatives on ^{3}H-thymidine incorporation into MCF-7 cells was examined to obtain further information on the possible mechanism of action of this compounds. In this assay the IC_{50} of only the most active compounds in the proliferation assay were determined, i. e., PCACL 22, 8, 23 and 12 (Table 2, Figure 5). Most of the compounds tested showed a significant DNA synthesis inhibition with IC_{50} values smaller than 6 μM after 24 hours of cell treatment with the drugs. PCALC 23 was the less active compound in this assay (IC_{50} = 12.86 μM) (Table, Figure 5). The IC_{50} value in the tritium incorporation assay for PCALC 23 was different from the IC_{50} value obtained in the proliferation experiment, however this drug was tested just once and replicates of this assay must be done with this compound to confirm this first results. The analysis of the data obtained with the compounds PCALC 22 and 8 which had IC_{50} values of 3.25 and 5.23 μM in the ^{3}H-thymidine incorporation assay, respectively, showed that a lower concentration of this two drugs were necessary to inhibit DNA synthesis when compared to the proliferation assay. Further studies on these series of compounds are underway aiming the investigation of their mechanism of action. This future experiments consist of the SV40 DNA synthessome replication assay and effect of this compounds on the DNA synthesome associated DNA polymerase α, topoisomerase I and II.

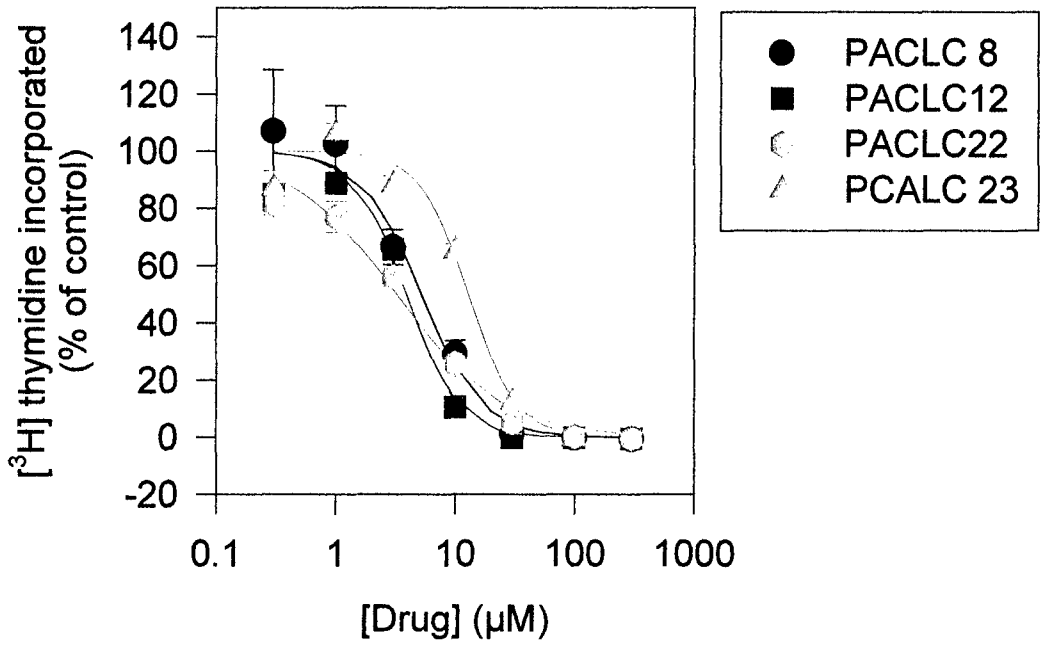

Figure 5: Effect of the 1,4-naphtoquinones derivatives on intact MCF-7 cell DNA synthesis after 24 hours of treatment with the drugs. Each point represents the average of at least one independent experiment in which four replicates determinations were taken; error bars represent the standard errors of the means. Cells grown and labeled in the absence of drug served as the controls (100 %) to which the drug treated cells were compared. The IC_{50} curves were determined mathematically based on the Hill coefficient.

KEY RESEARCH ACCOMPLISHMENTS

- investigated the antiproliferative activity of a new hydrazone series of compounds in breast cancer cells.

- investigated the antiproliferative activity of a new 1,4- naphtoquinone series of compounds in breast cancer cells.
- investigated the DNA synthesis inhibition activity of a new hydrazone series of compounds in breast cancer cells.
- investigated the DNA synthesis inhibition activity of a new 1,4-naphtoquinone series of compounds in breast cancer cells.
- performed experiment to verify if the human breast cancer DNA synthesome can serve as an *in vitro* model system for studying the mechanism of action of ribonucleotide reductase inhibitors.

REPORTABLE OUTCOMES

Abstracts

Macedo, L. F., Barreiro E. J., Malkas L. H., Albuquerque E. X., Hickey, R. (2000): Antiproliferative Activity of a New Series of Hydrazones Derivatives. Scientific Proceeding of the 91st Annual Meeting of the American Association for Cancer Research. 4157.

Macedo, L. F., Abdel-Aziz, W., Barreiro, E. J., Malkas, L. H., Hickey, R. (2001): Investigations on the Mechanism of Action of Novel Acyl-hydrazones and Sulfonylhydrazones Derivatives. Scientific Proceeding of the 92nd Annual Meeting of the American Association for Cancer Research. 431.

Papers

Da Silva, A. J. M., Buarque, C. D., Brito, F. V., Aurelian, L., Macedo, L. F., Malkas, L., Hickey, R., de Souza, D. V., Noźl, F., Murakami, Y. L. B., Silva, N. M. V., Melo, P. A., Caruso, R. R. B., Castro, N. G., Costa, P. R. R. (2001): Synthesis and preliminary Pharmacological Evaluation of New 1,4-Naphtoquinones Structurally Related to Lapachol. Manuscript in preparation.

Macedo, L. F., Barreiro, E. J., Malkas, L., Hickey, R. (2001): Antiproliferative properties and synthesis of a new series of hydrazone analogues. Manuscript in preparation.

CONCLUSIONS

- The hydrazones compounds LASSBio 466, 467, 276 and 273 inhibited cell proliferation and DNA synthesis in a significative manner being more potent than the RR inhibitor hydroxyurea.

• Analysis of the structure-activity relationships in the hydrazone series suggested some structure features that a responsible for the antiproliferative and DNA synthesis inhibition activity and can be used to design new other drugs with improved efficacy.
• Most of the new hydrazone drugs tested were unable to inhibit the cellular DNA synthetic apparatus (i. e., DNA synthesome) at concentrations up to 300 μM (LASSBio 273 at 300 μM showed a light inhibition) which is consistent with them being RR inhibitors since the deoxyribonucleotide biosynthesis enzymes are not a part of the DNA synthesome. The data obtained with araCTP in this assay confirmed results described previously (Abdel-Aziz, W. *et al.*, 2000).
• Our data suggested that at least two of the hydrazone derivatives , LASSBio 466 and 467, may be substantially more effective than hydroxyurea in current treatment protocols.
• The preliminary data obtained with the 1,4naphtoquinones derivatives are in agreement with them being topoisomerase inhibitors .
• The 1,4-naphtoquinone compounds PCALC 8, 12, 22 and 23 inhibited cell proliferation and DNA synthesis in a very significative manner.

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